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Evaluation of a commercial immunoassay for the detection of chlorfenapyr in agricultural samples by comparison with gas chromatography and mass spectrometric detection

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Abstract

A commercially available enzyme-linked immunosorbent assay (ELISA) kit with a high affinity monoclonal antibody was applied to residual analysis of insecticide chlorfenapyr in agricultural samples, and drawn a parallel between the ELISA and gas chromatography (GC) with mass spectrometry (MS). For standards prepared in water containing 5% (v/v) methanol, the sensitivity (I_{50} value), the dynamic range, and the limit of detection of the ELISA kit were 2.3, 1–10, and 0.1 ng/g, respectively. The used monoclonal antibody in the ELISA kit had a high selectivity. The ELISA kit was applied to the determination of chlorfenapyr in two kinds of fruits (apple and peach). The examination of the influence of these matrices on the reliability of the assay performance indicated that the ELISA could determine it in these samples near the regulation values in Japan simply by diluting the methanolic extract or by concentrating it, without any clean-up procedures. Recovery and precision of the proposed ELISA method were assessed by fortifying fruit samples with chlorfenapyr ranging from 0.05 to 1.5 μg/g. Mean recoveries were 94.2 and 90.3% for apple and peach, and coefficients of variation were below 16% in most cases. The results obtained from the proposed ELISA method correlate well the reference GC/MS method for both fruit samples ($r > 0.98$). These considerations make the ELISA kit very useful analytical tool for monitoring and regulatory programs, without the need of complex and expensive instrumentation.

Keywords: Chlorfenapyr; Pesticide; ELISA; Validation; Screening; Fruits

1. Introduction

The advantages of immunoassays (enzyme-linked immunosorbent assays, ELISAs) relative to other analytical techniques have been widely discussed by some researchers [1–3], and include the following: (1) low limit of detection, (2) high analyte selectivity (specificity), (3) high throughput of samples, (4) reduced sample preparation, (5) cost effectiveness for large numbers of samples, and (6) adaptability to field use. Moreover, no complex or sophisticated instrumentation is required and the use of toxic organic solvents is minimal. Hence, numerous immunoassays for various pesticides have been developed up to now as reviewed by Meulenberg et al. [1] and Shan et al. [4]. For screening purposes of residual pesticides in a wide variety of matrices, numerous ELISA kits have been developed and on sale as reviewed by Gabaldón et al. [5]. These kits are generally

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designed that users are easy of employment. Actually, the kit manufacturers or many reports on ELISAs for residual pesticide analyses usually propound that the ELISA techniques are easy for whom to operate and everyone can grasp the residual levels in samples without difficulty. However, these catchphrases are actually incompatible with the actual properties of the ELISA techniques. Since they are susceptible to physicochemical factors such as pH, ion strength or temperature, selectivity (cross-reactivity) of antibody used, organic solvents used in extraction or clean-up procedure, and matrix interferences coming from samples made up of complex constituents, the users may not necessarily be able to determine them without some trouble. So, these facts can act as a brake on the acceptance of the ELISA techniques for pesticide analyses and the promotion. Hence, the users should always keep the above-mentioned characteristics of the ELISA techniques in mind before use an ELISA kit. We have recently tested the performance of an ELISA kit which has been developed for a kind of neonicotinoid insecticide, imidacloprid, and reached the conclusions that the use of the ELISA kit could be effective as a screening purpose or a complementary analytical method to confirm positive results by chromatographic method for the residue monitoring in some crops [6,7].

Chlorfenapyr, 4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethylpyrrole-3-carbonitrile as an object of the present work was developed by American Cyanamid Co., and is a novel broad-spectrum insecticide–acaricide for control of various species of insects and mites, including those resistant to carbamate, organophosphate and pyrethroid insecticides and also chitin-synthesis inhibitors, in cotton, vegetables, citrus, and soy beans [8]. Chlorfenapyr is actually a pro-insecticide that is converted to an active metabolite in the midgut of the insects and mites. Once formed the metabolite uncouples oxidative phosphorylation by disrupting the proton gradient across mitochondrial membranes, thus affecting the ability of cells to produce ATP from ADP, which ultimately results in cell death and death of the organism. Gas chromatography (GC) equipped with nitrogen-phosphorus detector (NPD) [9,10] or electron capture detector (ECD) [9,11] is generally used as the best technique for chlorfenapyr analysis. Although the methods work well, they involve laborious extraction and clean-up procedures, and concentration steps are often needed to obtain the desired sensitivity. In the present work, the suitability of a recently developed ELISA kit for chlorfenapyr in Japan is evaluated. With the aims, the fundamental characteristics (sensitivity, dynamic range, and limit of detection), the selectivity, the influence of organic solvents on the assay performance, and the influence of the matrix interference on the reliability of the ELISA were investigated. Moreover, some appropriate extraction methods were examined to extract chlorfenapyr from agricultural samples as simply and rapidly as possible. Finally, chlorfenapyr concentrations in spiked fruit samples (apple and peach) were analyzed by the proposed ELISA technique.

2. Experimental

2.1. Chemicals and materials

Pesticide-grade chlorfenapyr and other standards (fenpyroximate, tebufenpyrad, fipronil, and fludioxonil) for cross-reactivity studies were purchased from Kanto (Tokyo, Japan) and Hayashi (Osaka, Japan), and were certified at least >98% pure. Tolfenpyrad (99.9% purity) was a gift from Otsuka (Tokushima, Japan). Ethiprole (96.2% purity) was a gift from Bayer CropScience (Ibaraki, Japan). Pesticide-grade organic solvents, diatomaceous earth, anhydrous sodium sulfate, and salts for preparation of assay buffer were from Wako (Osaka, Japan) and Kanto. Sep-Pak Florisil cartridges which were pre-packed with 1 g of Florisil were from Waters (Milford, MA, USA). The ELISA kits for chlorfenapyr (SmartAssay series) were purchased from Horiba Biotechnology (Kyoto, Japan). Water used in all experiments was purified with a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Samples

Apple and peach samples were purchased from a local market, and analyzed for incurred residue of chlorfenapyr by GC/mass spectrometry (MS). No incurred residue was detected.

2.3. Sample preparation for ELISA analysis

After these fruit samples were rinsed with water, and chopped, some known concentrations of chlorfenapyr were spiked to 5 g of each from stock solutions in methanol to make the appropriate concentrations, and then they were exposed to the chlorfenapyr for about 24 h prior to extraction.

For apple samples, 25 ml of methanol was added to each sample in 50 ml of disposable conical tube, and then the mixture was vigorously shaken by hand for 3 min. A portion of the methanolic extract was filtered through a disk type filter (Millex-HV syringe filter unit, 0.45 μm, Millipore). The filtrate extract was diluted 25-fold with 10 mM phosphate-buffered saline (PBS; 7.75 mM Na_2HPO_4, 2.25 mM KH_2PO_4, 154 mM NaCl, pH 7.2), and the diluted sample was then analyzed by ELISA without a further clean-up procedure. On the other hand, peach samples (5 g) were extracted with acetone (25 ml) with the same manner as above described. After shaking, the sample mixture was filtered to a 100 ml of round-bottomed flask through a funnel by suction. The tube and the residue on the funnel were washed with a small quantity of acetone, and it was also filtered to. The acetone in the filtrate was completely removed with a rotary evaporator, and then the residue was dissolved in PBS/methanol (9:1, v/v) up to 100 ml for ELISA analysis.

2.4. ELISA analysis

The chlorfenapyr kit consists of a split-type microtiter plate (12 strips, eight wells each) coated with an anti-

chlorfenapyr monoclonal antibody, two standard solutions (2 and 10 ng/g in water/methanol (9:1, v/v)), a horseradish peroxidase (HRP)-labeled conjugate (enzyme tracer), a color solution consisting of a substrate (hydrogen peroxide) and a chromogen (3,3′,5,5′-tetramethylbenzidine), a stopping solution (0.5 M H_2SO_4 solution), and a washing solution. Absorbances were measured with a SmartReader MPR-01 (Horiba Biotechnology) in a single wavelength mode (450 nm). Each well was washed with a washing solution containing detergent using a MiniLab washer (Lifetec, Saitama, Japan). The attached standard solutions were used by dissolving in 1 ml of water/methanol (9:1, v/v). Another stock solution (1000 mg/kg) was prepared by dissolving suitable amount of chlorfenapyr standard in methanol. Some working standard solutions for ELISA were also prepared with water/methanol (9:1, v/v) or PBS/methanol (9:1, v/v) for the evaluation of the ELISA kit by using the stock solution. Standard solutions and extracts from fortified samples were analyzed according to the following procedure: 150 μl of either standard solution or sample extract, properly diluted with water, was added to borosilicate glass tubes, followed by 150 μl of an enzyme tracer solution. After the well-mixed solutions (100 μl/well) were added to the above microtiter plate in duplicate at least, the well were covered with plate seal to minimize evaporation and incubated at ambient temperature for 1 h. After incubation, the seal was removed, and the wells were washed with a washing solution four times and tapped dry. The amount of the bound enzyme tracer is revealed by the addition of a substrate solution (100 μl/well) for color development. The wells were incubated for 10 min at room temperature keeping out the direct rays of the sun. After the incubation period was complete, stopping solution (100 μl/well) was added to each well. Quantitation was based on the optical density of the wells at 450 nm.

2.5. Sample preparation for GC/MS analysis

Chopped fruit samples (20 g) spiked with chlorfenapyr were vigorously shaken with 100 ml of acetone for 30 min, and then the sample mixture was filtered to a 300 ml of round-bottomed flask through a funnel spread with diatomaceous earth by suction. The residue on the funnel was similarly treated with 50 ml of acetone by above-mentioned manner. After the final volume of all filtrates was adjusted to 200 ml by addition of acetone, an aliquot of extract (100 ml) was concentrated to about 20 ml with a rotary evaporator. The concentrated solution was transferred to a 250 ml of separatory funnel and was vigorously shaken with adding 100 ml of 5% (w/v) NaCl solution and 50 ml of *n*-hexane for 5 min. Another 50 ml of *n*-hexane was added to the aqueous phase and the mixture was shaken for 5 min. After the organic phase was dehydrated with 20 g of anhydrous Na_2SO_4, it was concentrated with a rotary evaporator, and then was done with a gentle stream of nitrogen. The residue was dissolved in 1 ml of *n*-hexane, and then was loaded to Sep-Pak Florisil cartridge, which was washed and conditioned with 5 ml of *n*-hexane in advance. The applied cartridge was washed with 20 ml of *n*-hexane, and then the chlorfenapyr was eluted with 30 ml of *n*-hexane/diethyl ether (17:3, v/v). After the eluate from the cartridge was concentrated with a rotary evaporator, the residue was dissolved in *n*-hexane up to 5 ml for GC/MS analysis.

2.6. GC/MS analysis

A Hewlett-Packard 6890 gas chromatograph was used to confirm the identity of chlorfenapyr. It was fitted with a mass-selective detector Hewlett-Packard 5973, and was equipped with a Hewlett-Packard 7683 autosampler and Hewlett-Packard 7683 split/splitless injector with electronic pressure control. Residual chlorfenapyr was analyzed with a DB-5MS fused-silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The column oven temperature was held at 90 °C for 1 min, then programmed at 10 °C/min to 270 °C, and held for 5 min. The total run time was 24 min. Helium was used as the carrier gas with flow rate of 1.0 ml/min. The temperatures of the ion source and the quardrupole were 230 and 150 °C, respectively. The temperature of the injector was 250 °C, and the sample (2 μl) was injected in the splitless mode. The mass spectrometer was operated in the electron ionization mode (EI, 70 eV), and the analysis was carried out with selected ion monitoring (SIM) mode in which two characteristic ions for chlorfenapyr were monitored, m/z = 59 and 247.

3. Results and discussion

3.1. Fundamental characteristics of ELISA

Typical standard curve of the chlorfenapyr ELISA with the self-made standard solutions prepared in water/methanol (9:1, v/v) (5% (v/v) methanol in the each well) based on triplicates is shown in Fig. 1. According to the kit manufacturer, the dynamic range of the ELISA kit is from 1 to 5 ng/g. The dynamic range of the ELISA, defined by concentrations yielding 20–80% inhibition of the maximum signal (I_{20}–I_{80}), experimentally estimated based on the self-made standard solutions was from 1 to 10 ng/g. The chlorfenapyr concentrations that reduced maximum absorbance by 50% (I_{50} value) estimated with two standard solution series was 2.3 ng/g. On the other hand, the limit of detection, calculated as the chlorfenapyr concentration that reduced maximum absorbance by 10% (I_{10} value), was 0.1 ng/g. Moreover, as shown in Fig. 1, the linearity of the standard curve based on the kit-assembled standard solutions (slope = −0.466) (data not shown) agreed with that based on the self-made standard solutions (slope = −0.450). The experimentally estimated dynamic range (1–10 ng/g) was somewhat wider than that suggested by the kit manufacturer (1–5 ng/g).

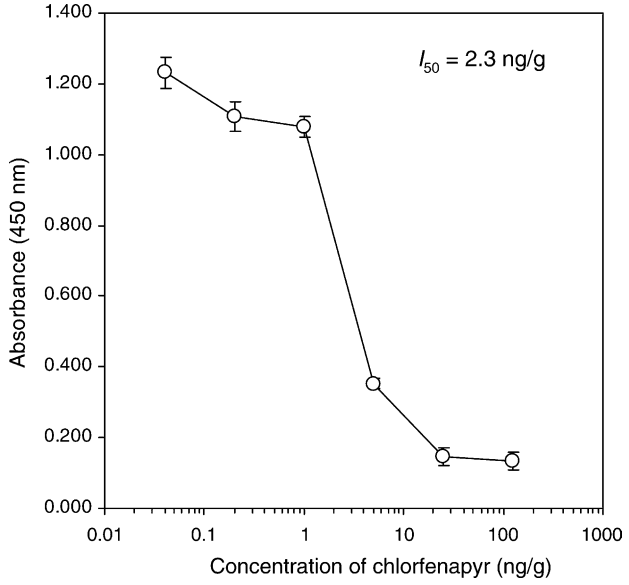

Fig. 1. ELISA inhibition curve for chlorfenapyr produced with the self-made standard solutions, which were prepared in water/methanol (9:1, v/v). Each point is the average of triplicate determinations. Error bars indicate ±SD about the average absorbance.

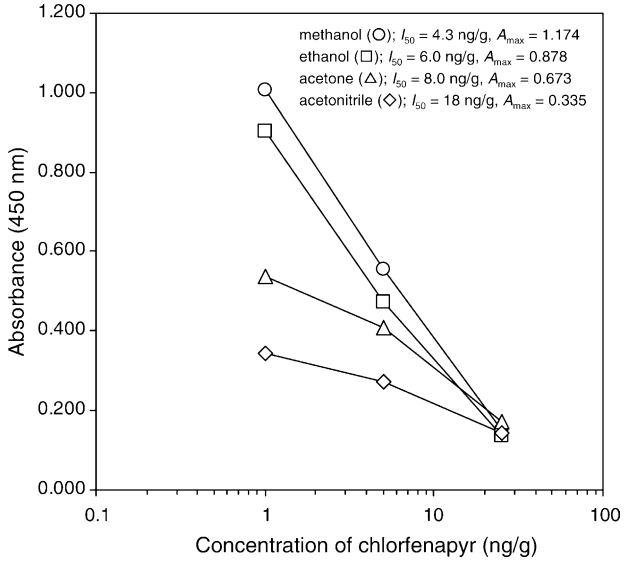

Fig. 2. Selection of organic solvents for extraction procedure from agricultural samples, and their influence on the color development and the sensitivity of the ELISA. The data are the average of two replicates. The final concentration of each solvent (in the well) is 5%.

3.2. Cross-reactivity

Because of the lack of the ability to identify compounds in the ELISA techniques, it is essential to grasp the cross-reactivity of antibody used in the ELISA kit. Cross-reactions can affect analytical results either by indicating that the target compound is present when it is not or by elevating the predicted concentration of the target compound when both the target and one or more structurally similar compounds are present. So, cross-reactivities were calculated as the ratio of the I_{50} value of chlorfenapyr to the I_{50} values of test compounds and expressed as a percentage. The monoclonal antibody used showed no cross-reaction against examined analogues. So, the antibody was highly specific to chlorfenapyr.

3.3. Influence of organic solvents on assay sensitivity

Chlorfenapyr has the property that it is practically insoluble in water [8]. So, it should be used water-miscible organic solvents in which chlorfenapyr is soluble [8] such as acetone [9,10], acetonitrile, or methanol to efficiently and quantitatively extract it from agricultural samples. However, the application of ELISAs based on antigen–antibody interaction is hindered by the low water solubility of the compounds to be detected. The antibodies are usually inactive in high concentrations of organic solvents, and generally ELISAs can only tolerate up to about 10% (v/v) of organic solvent in aqueous solution as seen in several reports related with ELISA method for residual pesticide analyses [6,7,12–14]. Accordingly, the influence of organic solvents used on the specific interaction between the analyte and the antibody should be elucidated. So, first of all, the influences of four kinds of organic solvents on the assay performance were assessed, and then the most suitable solvent for extraction procedure and assay performance was selected based on the variation of I_{50} values which indicate the sensitivity of the ELISA kit and A_{max} values which index the speed of color development. As shown in Fig. 2, the A_{max} values in the presence of acetonitrile and acetone were much lower than those in the presence of two kinds of alcohols. On the other hand, the lowest I_{50} value was found at methanol, which is 4.2 times lower than that at acetonitrile, 1.9 times lower than that at acetone, and 1.4 times lower than that at ethanol, respectively. So, methanol, which has little interference with the sensitivity and the color development at 5% (v/v), was selected as the most suitable organic solvent for the ELISA kit.

In next stage, the influence of selected methanol on the assay performance was investigated by preparing serial standard solutions in water containing various amounts of it (1, 5, 10, 20, and 30% (v/v) in water as final concentrations in the wells). As shown in Fig. 3, the A_{max} value decreased with increasing the concentration, especially >20% (v/v). On the other hand, the I_{50} values in water containing 1 and 30% (v/v) as final concentration were about two times higher than those in other contents. As chlorfenapyr is practically insoluble in water [8,10], it is necessary to use a small amount of methanol in which it is soluble, in water or assay buffer to reduce assay variability by reducing binding of the analyte to surfaces of the plate. Thus, these knowledge indicated that it was desirable for the ELISA kit to use methanol as extractant and to control the final concentration in each well 5% (v/v) or less.

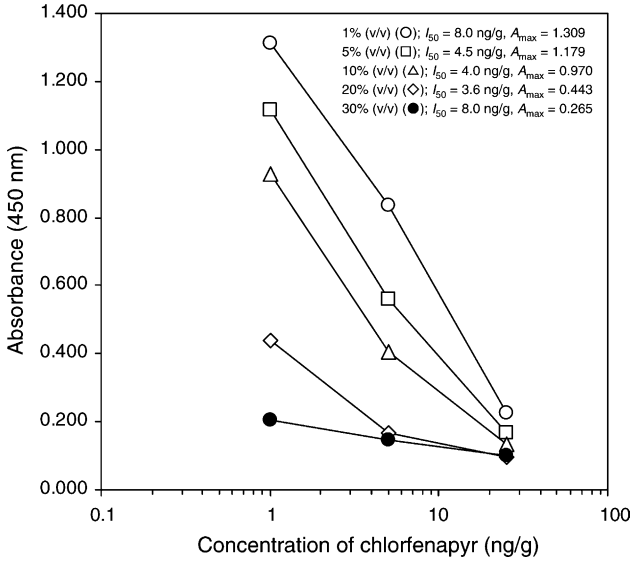

Fig. 3. Influence of concentration of methanol, which was optimized as a suitable organic solvent for extraction procedure on the color development and the sensitivity of the ELISA. The data are the average of two replicates.

3.4. Matrix interference and optimization of assay diluent for fruit extract

Apples and peaches were chosen as model matrices to evaluate the suitability of the ELISA kit for the analysis of fruits. Apart from their relevance for the target analyte under the present study, these matrices were chosen because they do not present interferent compounds in the chromatographic area of interest, thus allowing an optimal quantification of the analyte (Fig. 4). It is well known that though the ELISA techniques make good use of specific bioreaction, antigen–antibody interaction, they are generally susceptible to interference to the interaction coming from various components such as tannins, polyphenols, sugars, or lipids in agricultural or food samples, that is, matrix interference, when applying them to actual samples. As seen in several reports [2,15], the most simplest and easiest method for removal of sample matrix interference in immunoassays is to dilute an extract from a sample with a correct diluent such as water [6,7] or buffer [14,16,17]. In order to assess and correct the matrix interference caused by these two kinds of fruit matrices, they were initially extracted with selected methanol, each methanolic extract was diluted with water, and the absorbance-concentration plot for a range of standards (between 1 and 25 ng/g) compared with that of standards prepared in water/methanol (9:1, v/v) (Since the standards for control curve are mixed with equal volume of enzyme tracer solution, the final concentration of methanol in each standard is 5% (v/v)).

As shown in Fig. 5, the standard curves for peach extracts which were generated by diluting at two- and three-fold with water were significantly different from the standard curve produced in water/methanol (9:1, v/v). It was clear that the matrix interference coming from peach samples markedly

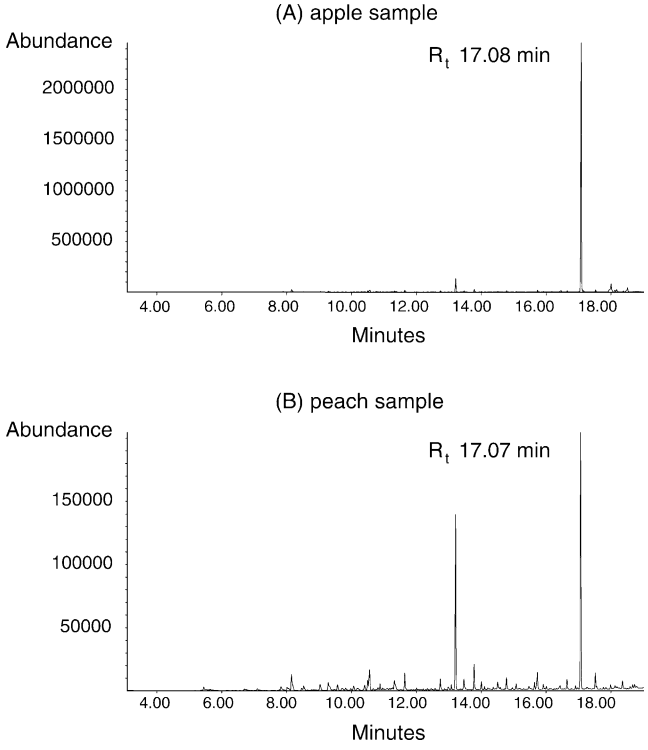

Fig. 4. GC/MS chromatograms of extracts of (A) apple and (B) peach samples fortified with chlorfenapyr at 1 μg/g (for apple) and 0.1 μg/g (for peach). Analytical conditions for GC/MS are described in Section 2.6.

appeared. However, it suggested that it could attribute the negative result not only to the matrix interference but also to the excess of the final concentration of methanol in each well. When taking account of the following matters (1) the regulation value of chlorfenapyr for peach is set up 0.1 μg/g (ppm) in Japan [9], and (2) the sensitivity of the ELISA kit was 2.3 ng/g as described in Section 3.1, the above-mentioned dilution factor (two- or three-fold) was at the limitation on avoidance of the matrix interference. Hence, it thought that it is difficult to analyze the chlorfenapyr in peach samples with the ELISA kit by only dilution of the extract with water. So, a concentration step for peach extract was added to the pre-treatment procedure for peach samples. After methanol and acetone were used as extractants, and each extract, which was obtained by suction filtration, was evaporated, the residues were diluted at 5-, 6-, 7.5-, and 20-fold with water/methanol (9:1, v/v) or PBS/methanol (9:1, v/v). When diluting with water/methanol (9:1, v/v), the standard curves, which were generated with four kinds of dilution factor, somewhat shifted to left side at the area of the lower concentration (data not shown). On the other hand, when acetone was used as extractant and the concentrated extract was diluted at 20-fold with PBS/methanol (9:1, v/v), the standard curve generated in peach extract agreed with the one generated in PBS/methanol (9:1, v/v), as shown in Fig. 5. The standard curves which were obtained from concentrated extracts with methanol as extractant slightly disagreed with the one generated in PBS/methanol (9:1, v/v) at the area of

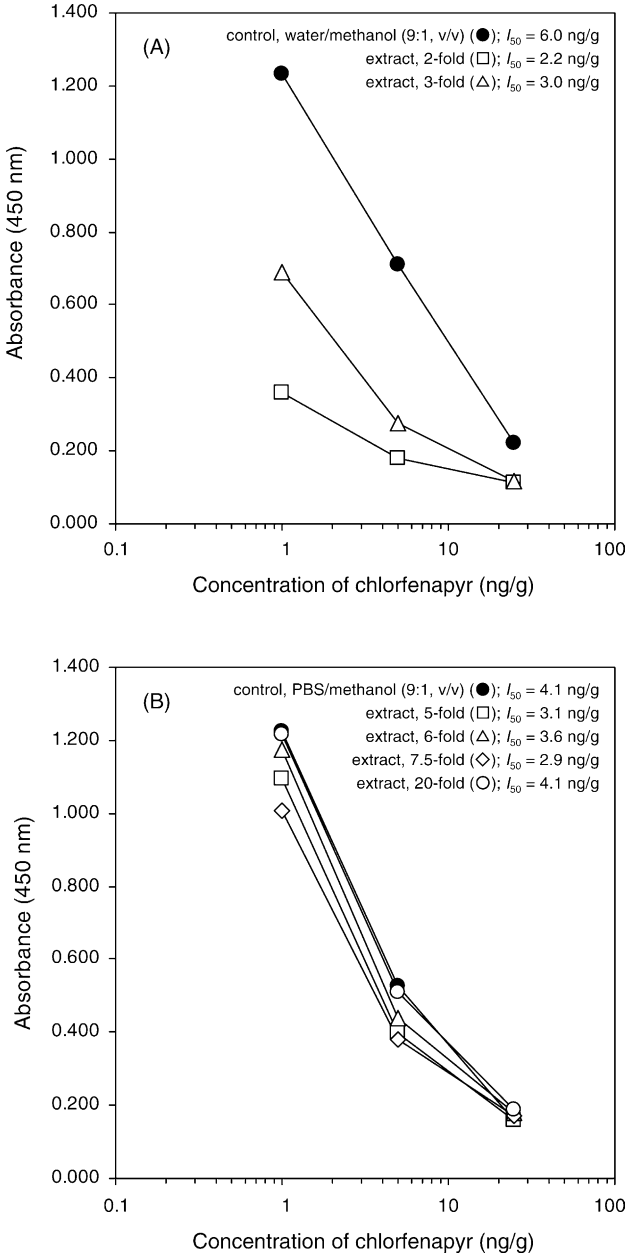

Fig. 5. Influence of peach matrix interference on the assay performance and the removal by concentration and dilution with PBS containing 10% (v/v) methanol (5% (v/v) methanol in the each well).

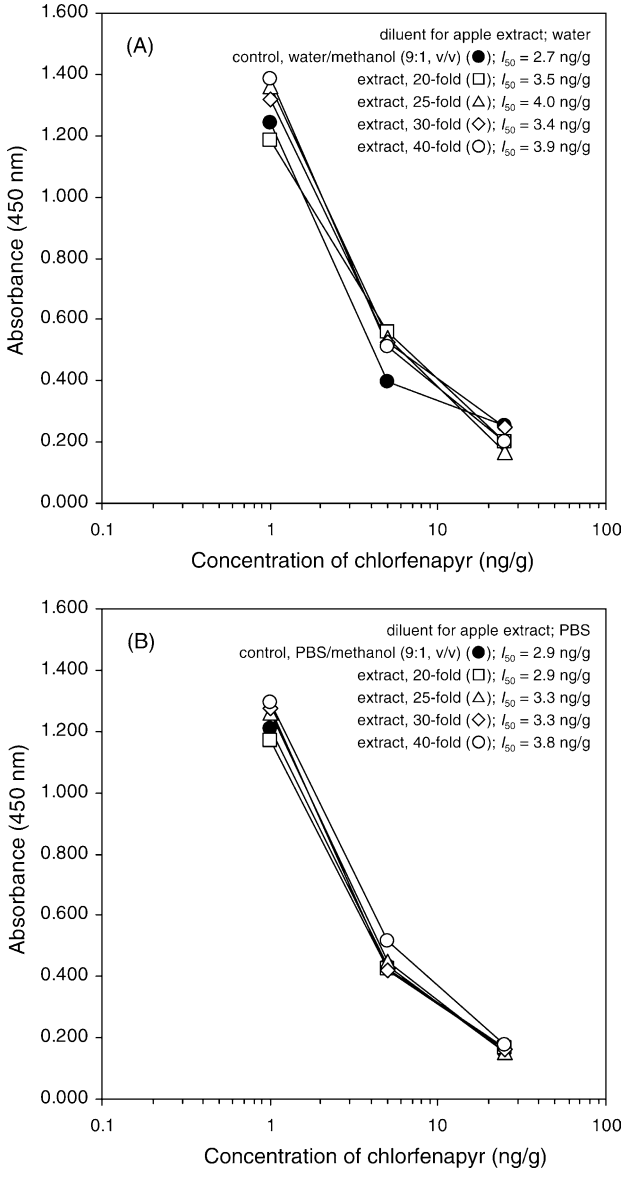

Fig. 6. Influence of apple matrix interference on the assay performance.

the lower concentration (data not shown). Accordingly, the chlorfenapyr in peach sample was analyzed by concentrating acetone extract and diluting at 20-fold with PBS/methanol (9:1, v/v) as the best approach to avoid the peach matrix interference.

For apple samples, since the regulation value is set up 1 μg/g (ppm) [9], it thought that it could be possible to overcome the matrix interference only giving dilution with proper diluents. So, methanolic extract was diluted at 20-, 25-, 30-, and 40-fold with water or PBS, and the obtained standard curves drew a parallel between them and the standard curves generated in two diluents as above-described. As shown in Fig. 6, although the matrix interference was improved when diluting with water, the use of PBS served to be the avoidance. Thus, PBS was selected as a best diluent and the dilution factor was set up 25-fold for the chlorfenapyr in apple sample with the ELISA kit.

Since PBS was selected as diluent, and the fundamental characteristics of the ELISA kit were assessed based on water in Section 3.1, they were evaluated by using PBS afresh (Fig. 7). Each analytical parameter was as follows: I_{50}, 3.0 ng/g; dynamic range, 1.1–14 ng/g; limit of detection, 0.5 ng/g; slope of standard curve, −0.448, respectively. The use of PBS as diluent was no changes of several parameters in the ELISA kit when using water.

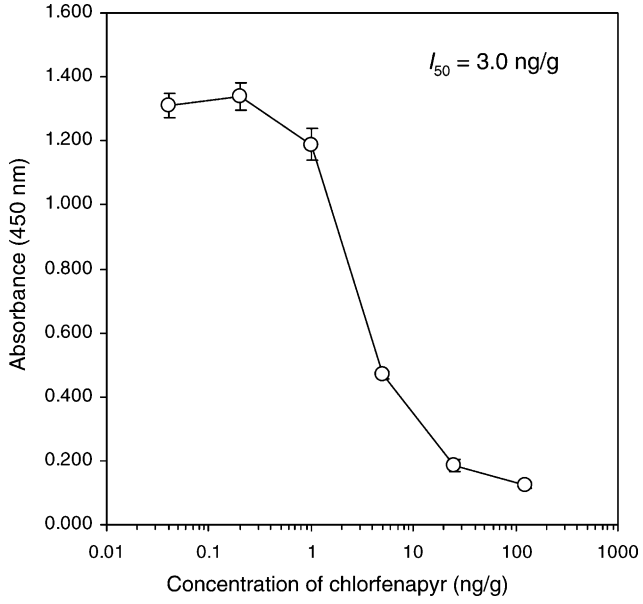

Fig. 7. Typical standard curve generated in PBS containing 10% (v/v) methanol (5% (v/v) methanol as final concentration in each well). Each point is the average of quadruplicate determinations. Error bars indicate ±SD about the average absorbance.

3.5. Extraction method for ELISA

The advantages of the immunochemical techniques for pesticides in agro-environmental matrices as described by Skerritt [18] and Ellis [19] are highly elicited by simplifying the pre-treatment, and moreover, extraction procedure should be rapid and easy when finally fixing one's eyes on an on-site screening purpose. So, four extraction methods were trailed and the most suitable method for an on-site screening purpose on the basis of the data (recovery and reproducibility) was selected in this stage. For the study, the peach samples fortified with chlorfenapyr at 0.1 μg/g and the apple samples done with it at 1 μg/g were extracted by four different methods as shown in Table 1. The recovery values for apple samples obtained from all methods were almost equality (>96%). On the other hand, the value for peach samples obtained from the simplest method, hand-shaking for 3 min among the examined method was reasonably efficient, quantitatively extracting >88% of the incurred chlorfenapyr in them with good reproducibility. Furthermore, because the method does not require the instruments for extraction process, it is thought that it could be the most suitable for the above-mentioned purpose.

3.6. Recovery analysis in spiked fruit samples

Fortifying matrix samples with several amounts of analyte is a common practice to perform a preliminary evaluation of analytical assay reliability. Hence, two fruit samples were fortified at several concentrations of chlorfenapyr covering the dynamic range estimated from Fig. 7, and the fortified samples were analyzed based on the optimal conditions in which no significant matrix interference were observed described in Section 3.4. As given in Table 2, the mean recovery data for these fruit samples at four levels of fortification were 94.2% (varying from 91.7 to 96.7%) and 90.3% (varying from 85.3 to 93.3%) for the apple and peach samples, respectively. Concerning the reproducibility, average intra-assay coefficients of variation were 8.7 and 6.4% for the apple and peach samples, respectively. It was thought that the ELISA kit is very useful to analyze if residue in a sample is against the regulation value for both fruit samples or not.

3.7. Method comparison between proposed ELISA and reference GC/MS

Linear regression analysis was applied to data provided by the ELISA method for nonpurified samples versus those provided by the reference GC/MS method for purified samples as shown in Fig. 8. Despite the fact that the comparison was established on samples subjected to different pretreatment, the ELISA results well-correlated with the GC/MS results ($r = 0.9891$ for apple samples and $r = 0.9987$ for peach samples, respectively). The only significant discrepancy between results were the slope of the linear regression analysis of

Table 1
Extraction efficiency of various techniques and selection of extraction method for the ELISA[a]

Matrix	Extraction method	Detected concentration (μg/g)	Average recovery (%, $n = 3$)	Coefficient of variation (%)
Apple	Hand-shaking for 3 min	1.0 ± 0.050	100.0	5.0
	Mechanical shaking for 15 min	1.0 ± 0.066	100.0	6.6
	Ultrasonication for 15 min	0.99 ± 0.052	99.2	5.2
	Mechanical shaking for 30 min[b]	0.97 ± 0.144	96.7	14.9
Average			99.0	
Peach	Hand-shaking for 3 min	0.088 ± 0.007	88.0	7.9
	Mechanical shaking for 15 min	0.14 ± 0.018	138.7	13.0
	Ultrasonication for 15 min	0.080 ± 0.004	80.0	5.0
	Mechanical shaking for 30 min[b]	0.076 ± 0.004	76.0	5.3
Average			95.7	

[a] The apple samples fortified with chlorfenapyr at 1 μg/g and the peach samples done at 0.1 μg/g were extracted with each proposed method, respectively. Data are the average of three determinations performed on the same day.
[b] The method is recommended by the kit manufacturer.

Table 2
Recovery of the analyte chlorfenapyr fortified to fruit samples with the ELISA[a]

Matrix	Fortified level (μg/g)	Detected concentration (μg/g)	Average recovery (%, $n = 3$)	Coefficient of variation (%)
Apple	0.1	0.092 ± 0.014	91.7	15.7
	0.5	0.48 ± 0.038	96.7	7.9
	1.0	0.94 ± 0.080	94.2	8.5
	1.5	1.4 ± 0.038	94.4	2.7
Average			94.2	8.7
Peach	0.05	0.043 ± 0.002	85.3	5.4
	0.1	0.093 ± 0.006	93.3	6.5
	0.15	0.14 ± 0.008	90.7	5.9
	0.2	0.18 ± 0.014	92.0	7.8
Average			90.3	6.4

[a] Data are the average of three determinations performed on the same day.

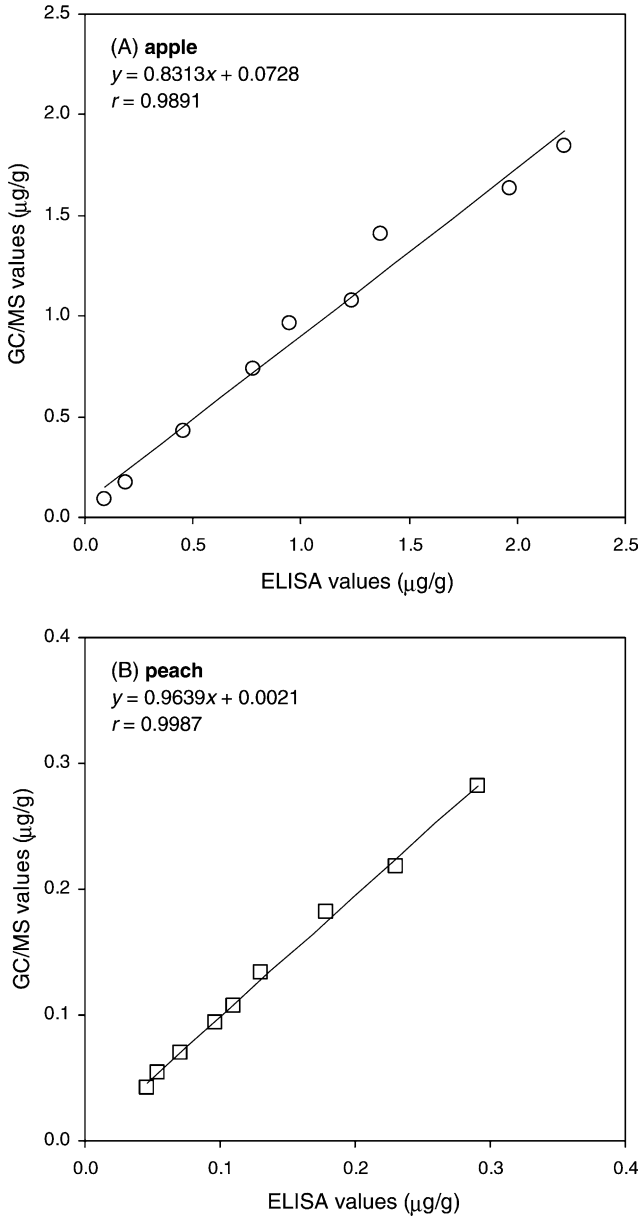

Fig. 8. Correlation between the proposed ELISA method and the reference GC/MS method for fruit samples fortified with chlorfenapyr.

apple samples, which was <1.0. The result is probably due to chlorfenapyr losses incurred as a consequence of sample clean-up procedures or evaporation steps (mean recovery of GC/MS; 91.3% for apple samples). Accordingly, the proven ability of the ELISA kit to accurately analyze chlorfenapyr in nonpurified fruit samples entails an undoubted practical advantage over methods requiring troublesome sample clean-up procedures.

4. Conclusions

Results in the present work clearly prove that the ELISA kit herein assessed are able to analyze chlorfenapyr in apple and peach samples at levels of regulation values in Japan with accuracy and precision comparable to those obtained with the reference GC/MS method. The main advantage of the reported ELISA method is the possibility to perform direct and accurate measurements of fruit samples without any clean-up procedures. For apple samples, the choice of a proper dilution factor minimized matrix interference and elicits many advantages of immunochemical techniques. On the other hand, the analysis in peach samples needs to be concentration step of extract to be surely analyzed in the neighborhood of the regulation value for peach and to overcome the matrix interference. So, the residual analysis in peach samples with the ELISA may be unsuitable to the on-site screening; nevertheless, the proposed ELISA method can be evidently more rapid and simple than the conventional analytical methods. Practically, with standard solutions for the calibration curve and samples run at least in duplicate, about 40 samples or more can be screened on a set of the ELISA kit. Since the pre-treatment for the proposed ELISA analysis finishes only extraction and dilution procedures for apple samples and additionally concentration for peach samples, about 15 min for apple samples and about 45 min for peach samples are required per one sample, respectively. Overall, the relatively low cost (about $960 per one kit), minimal sample pre-treatment and organic solvent waste, rapid analysis time, high sample throughput, and ease of use of the mi-

crotiter plate ELISA make it well suited for adaptation to screening for chlorfenapyr in fruit samples. Before the proposed ELISA kit can be routinely employed for regulatory compliance monitoring, however, further investigation is required to extend the application of the ELISA to further fruits and vegetables.

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